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RESEARCH ARTICLES

In vitro salinity priming modulates photosynthetic acclimation in *Ananas comosus* with limited growth recovery

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Abstract

Salinity is a major abiotic constraint limiting pineapple (*Ananas comosus*) growth and productivity, and strategies that improve stress acclimation during early developmental stages may enhance plant performance under adverse conditions. This study evaluated whether in vitro salinity priming could modulate subsequent physiological and growth responses of pineapple seedlings exposed to severe *ex vitro* salt stress. Seedlings were subjected to salinity conditioning during the in vitro phase and subsequently acclimatized under greenhouse conditions, with or without exposure to 500 mM NaCl. Growth, gas exchange, chlorophyll fluorescence, and photosynthetic pigment content were assessed. Severe salinity reduced net CO_2 assimilation, stomatal conductance, transpiration, and biomass accumulation in both primed and non-primed plants. However, primed seedlings showed partial physiological acclimation, including a less pronounced decline in apparent carboxylation efficiency and maintenance of pigment concentrations under salinity. Contrasting *Ci/Ca* responses suggested differences in the balance between stomatal and non-stomatal limitations depending on priming history. Despite these physiological adjustments, priming did not improve biomass accumulation and was associated with reduced plant size even under non-saline conditions. These findings indicate that in vitro salinity priming can alter subsequent physiological responses in pineapple, but under the severe salinity imposed here, these adjustments were insufficient to promote growth recovery, suggesting a decoupling between selected physiological traits and biomass performance.

Keywords Chlorophyll fluorescence · Gas exchange · In vitro culture · Salinity priming

Introduction

Soil salinity is among the most important abiotic factors limiting agricultural productivity and currently affects substantial portions of cultivated land worldwide (Murtaza et al. 2025). In *Ananas comosus* (pineapple), salt exposure triggers both osmotic and ionic constraints that impair vegetative development and reduce productivity (Da Silva et al. 2025). These detrimental effects are largely associated with disturbances in cellular ion balance and water relations, which compromise fundamental metabolic processes necessary for plant growth. At the physiological level, salinity disrupts water balance and photosynthetic activity, decreases photochemical efficiency and relative water content, destabilizes membrane integrity, promotes electrolyte leakage and potassium loss, and accelerates the breakdown of total photosynthetic pigments (Zhang et al. 2025; Saleem et al. 2025).

Although substantial progress has recently been made in the development of in vitro propagation and regeneration protocols for *A. comosus*, expanding its biotechnological applications (Ranjan et al. 2026), improving salt tolerance in this species remains an important challenge. In this context, in vitro culture provides a valuable experimental

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platform for the precise application of controlled salinity treatments, enabling the investigation of adaptive physiological responses and the induction of stress priming mechanisms that may contribute to improved stress resilience. Within this framework, salinity priming has gained attention as a strategy in which exposure to moderate salt stress induces physiological and molecular adjustments capable of enhancing plant performance during later stress events, a phenomenon linked to stress memory (Pissolato et al. 2024).

The controlled nature of in vitro systems allows precise manipulation of stress intensity and exposure duration, facilitating the induction of a primed physiological state that may persist after acclimatization (He et al. 2022). In *A. comosus*, previous studies suggest that physiological conditioning during in vitro culture can influence plant performance after transfer to *ex vitro* conditions, highlighting the broader potential of in vitro approaches for improving stress resilience (Melo et al. 2011). However, it remains unclear whether in vitro salinity priming specifically can confer persistent physiological benefits that translate into integrated improvements in growth and photosynthetic performance during subsequent salinity exposure.

Addressing this issue is critical because enhanced physiological acclimation does not necessarily translate into superior plant growth. Stress tolerance mechanisms require energetic and metabolic investment, which may redirect resources from biomass accumulation to protective processes under adverse conditions (Zhao et al. 2020; He et al. 2022). Therefore, evaluating whether priming improves stress performance without compromising growth is essential for determining its functional and agronomic relevance.

Accordingly, this study evaluated the effects of in vitro salinity priming on growth, photosynthetic performance, and pigment stability in *A. comosus* plants exposed to salt stress after acclimatization. We hypothesize that in vitro salinity conditioning promotes improved physiological acclimation during subsequent salt exposure, contributing to greater photosynthetic stability, but that these protective adjustments may not necessarily translate into enhanced biomass accumulation due to the energetic and metabolic costs associated with maintaining stress defense mechanisms.

Materials and methods

Plant material and in vitro salinity priming

The experiment was with *Ananas comosus* cv. Pérola shoot previously established in vitro, which were successively subcultured 60 mL of Murashige and Skoog (PhytoTechnology®) medium containing 30 g L^{-1} sucrose (Dinâmica®), 100 mg L^{-1} myo-inositol (Sigma-Aldrich®), 4 µM 6-benzyladenine, and 2 µM naphthaleneacetic acid. The culture medium was solidified using 2 g L^{-1} phytagel, its pH adjusted to 5.7 ± 0.1 prior to sterilization, followed by autoclaving at 121 °C under 1.5 atm for 15 min.

Shoots approximately 2 cm long were subjected to two NaCl treatments: 0 mM (control) and 80 mM (saline preconditioning). Every 30 days, the plants were subcultured in a new culture medium supplemented with the respective NaCl concentrations (0 or 80 mM), totaling 90 days of culture (Carretero et al. 2007). Cultures were kept under controlled environmental conditions (25 ± 2 °C, 60% RH, 16-h photoperiod, and irradiance of 100 µmol m^{-2} s^{-1} supplied by white LED lamps).

Each treatment consisted of five replicates, with two explants per flask, and each flask considered as one experimental unit. At the end of the experimental period, growth and chlorophyll fluorescence were evaluated. Data were assessed by comparing control and NaCl-treated plants using a two-tailed t-test ($P \leq 0.05$ and $P \leq 0.01$).

Ex vitro salinity exposure

Following the in vitro phase, plantlets were transferred to 500 mL pots containing 450 g of washed sand and acclimatized under greenhouse conditions prior to the onset of salinity treatment. The experiment was arranged in a completely randomized design following a 2 × 2 factorial scheme, combining two in vitro conditions (0 and 80 mM NaCl) with two ex vitro salinity levels (0 and 500 mM NaCl). This design resulted in four treatment groups: unprimed control (0/0 mM), unprimed salinized (0/500 mM), primed control (80/0 mM), and primed salinized (80/500 mM). Each treatment consisted of 11 replicates, with one plant per pot considered as the experimental unit.

The concentration of 500 mM NaCl was selected to impose a severe salinity stress under controlled conditions. Previous studies indicate that 400 mM NaCl is sufficient to induce severe stress in pineapple without causing immediate lethality (Zhou et al. 2024).

Plants were maintained for 35 days under greenhouse conditions (28 ± 3 °C, 60% relative humidity, 12-h photoperiod, and irradiance of approximately 1075 µmol m^{-2} s^{-1}). Salinity treatments were established by daily irrigation with 100 mL of the respective NaCl solution. Irrigation was standardized across treatments, with drainage observed. Fertigation was applied at 7, 14, and 21 days after transplantation, with 100 mL per pot using Plantpar® fertilizer (Indústria e Comércio de Fertilizantes LTDA, Umuarama, PR, Brazil). According to the manufacturer's recommendation, the fertigation solution was prepared with 4.2 g of Plantpar Red Flex and 4.2 g of Plantpar Blue Flex per 10 L of water. Plantpar Red Flex contains N (9.0%), Mg (0.6%), S

(3.0%), P_2O_5 (9.0%), K_2O (37.0%), and the micronutrients Mn (0.048%), Cu (0.031%), Co (0.002%), Zn (0.019%), B (0.048%), Mo (0.009%), Fe (0.148%), and Ni (0.006%), while Plantpar Blue Flex contains N (13.0%), Ca (15.5%), and Mg (2.85%). Control plants received the same irrigation volume and fertigation schedule, but without NaCl addition.

At the end of the experimental phase, growth, gas exchange, chlorophyll fluorescence, and pigment content were evaluated. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Bartlett's test. Since the assumptions were met, no data transformation was required. Data were then subjected to two-way analysis of variance (ANOVA) in a 2 × 2 factorial design (priming × salinity), and treatment means were compared using Tukey's test at $P \leq 0.05$. All statistical analyses were performed using the Genes software (Cruz 2016).

Chlorophyll fluorescence and gas exchange

Chlorophyll fluorescence measurements were performed on leaf D using a portable chlorophyll fluorometer (Pocket PEA, Hansatech Instruments Ltd., King's Lynn, UK), which records fast chlorophyll fluorescence transients (OJIP). Before measurements, leaves were dark-adapted for 30 min using leaf clips to ensure full reoxidation of QA and complete stabilization of PSII reaction centers. The following fluorescence parameters were obtained: initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), maximum quantum efficiency of PSII (Fv/Fm), the ratio of variable to initial fluorescence (Fv/F_0), density of active reaction centers per absorption (RC/ABS), and the performance index (PI).

Gas exchange was evaluated between 06:30 and 08:00 h using an open-flow infrared gas analyzer (LI-6400XT, Li-Cor, Lincoln, NE, USA). Because *A. comosus* is a CAM species, preliminary prescreening measurements were performed to determine a time window in which stomata were consistently open under our experimental conditions, and the early morning period was selected accordingly to allow standardized comparison among treatments. Readings were obtained at a reference CO_2 concentration of 400 µmol mol^{-1}, air temperature maintained at approximately 29 °C, and a saturating photosynthetic photon flux density of 1000 µmol m^{-2} s^{-1} provided by an LED light source containing 10% blue wavelengths to ensure stomatal activation. Airflow, chamber humidity/VPD, inserted leaf area, and instrument correction parameters were maintained according to the manufacturer's standard operating conditions for the LI-6400XT. Although this approach enabled consistent treatment comparisons, it does not represent the full diel pattern of CO_2 assimilation characteristic of CAM metabolism, which should be considered when interpreting the results. The stabilization time inside the IRGA chamber was 3 min for all measurements.

Photosynthetic pigments

Photosynthetic pigments were determined from leaf discs obtained from the fifth fully expanded leaves. Samples were incubated in 3 mL of dimethyl sulfoxide (DMSO; Êxodo Científica®, Sumaré, SP, Brazil) and maintained in darkness for 48 h to ensure complete pigment extraction. Absorbance was subsequently recorded at 665, 649, and 480 nm using a UV–visible spectrophotometer (UV-M51, BEL Engineering, Monza, Italy), according to the method described by Pinheiro et al. (2025).

Growth analysis

Plant growth was evaluated based on the following parameters: shoot length, root length, rosette diameter, rosette base diameter, number of leaves, leaf area, and fresh and dry biomass of shoots and roots. Leaf area was determined using ImageJ® software from digital images of detached leaves captured against a contrasting background alongside a reference scale for calibration. The scale was calibrated within the software using the known reference measurement, and only fully expanded leaves without severe necrosis or deformation were included in the analysis to ensure consistent and reliable estimation of leaf area.

Relative leaf water content

Relative leaf water content (RLWC, %) was measured on the sixth fully expanded leaf. Five leaf discs (3 mm in diameter) were collected and immediately weighed to obtain the fresh mass (FM). Discs were then placed in plastic cups containing 8 mL of distilled water for 24 h to obtain the turgid mass (TM). Afterward, discs were oven-dried at 45 °C for 48 h to obtain the dry mass (DM) (Barr and Weatherley, 1962). Relative water content (RWC) was determined as described by Pinheiro et al. (2025).The relative water content was calculated using the following formula:

$$\mathrm{RLWC}(\%) = [(\mathrm{FM} - \mathrm{DM})/(\mathrm{TM} - \mathrm{DM})] \times 100$$

Results

Salinity reduces growth, photosynthetic efficiency, and pigment content in A. comosus plantlets cultivated in vitro

According to the F-test from the two-way ANOVA, all evaluated variables showed significant interaction effects between priming and salinity factors ($P \leq 0.05$).

Salinity-treated plants exhibited reduced growth, yellowing leaves, necrosis, and an atrophied root system. In contrast, control plants displayed higher shoot and root development with greener leaves during the first subculture. However, root length declined during the second and third subcultures even under non-saline conditions, which may reflect limitations inherent to the in vitro culture system, such as hormonal effects of the culture medium or physical constraints imposed by the cultivation flasks (Fig. 1).

Salinity affected the length of the aerial part, root length, number of leaves, and leaf area across subcultures. Root fresh and dry mass showed a differential response depending on the cultivation stage, with increases under 80 mM NaCl during the first cultivation, no significant changes in the second cultivation, and significant reductions in the third cultivation (Table 1). Plants grown under 80 mM NaCl showed a 33% reduction in growth and a 70% reduction in root length under salinity. Under 80 mM NaCl, root fresh and dry mass increased by 91% and 54%, respectively, during the first subculture, but decreased by 54% and 40%, respectively, during the third subculture. Salinity did not affect rosette base diameter; however, it reduced leaf number and leaf area, indicating a negative impact on leaf production and expansion. The effects on chlorophyll fluorescence parameters varied across subcultures, with progressively stronger reductions under salinity in later cultivation stages, indicating increasing impairment of PSII photochemical performance over time (Table 1).

Salinity significantly affected chlorophyll *a* fluorescence (Table 2), indicating stress-induced damage to the photosynthetic apparatus. Initial (F_0), maximum (F_m), and variable fluorescence (F_v) decreased by 21%, 45%, 51% and decreased the maximum quantum yield of PSII (Fv/Fm) from 0.77 to 0.61 under salinity. Salinity also reduced the density of active PSII reaction centers (RC/ABS) and the maximum primary efficiency of PSII (F_v/F_0) by 40% and 44%, respectively. The photosynthetic performance index (PI) decreased sharply from 2.33 to 0.91 (Table 2), indicating substantial loss of photosynthetic efficiency. Pigments were also reduced: chlorophyll a (54%), chlorophyll b (60%), carotenoids (50%), total chlorophylls (55%), and Chl/carotenoids (10%) (Table 2).

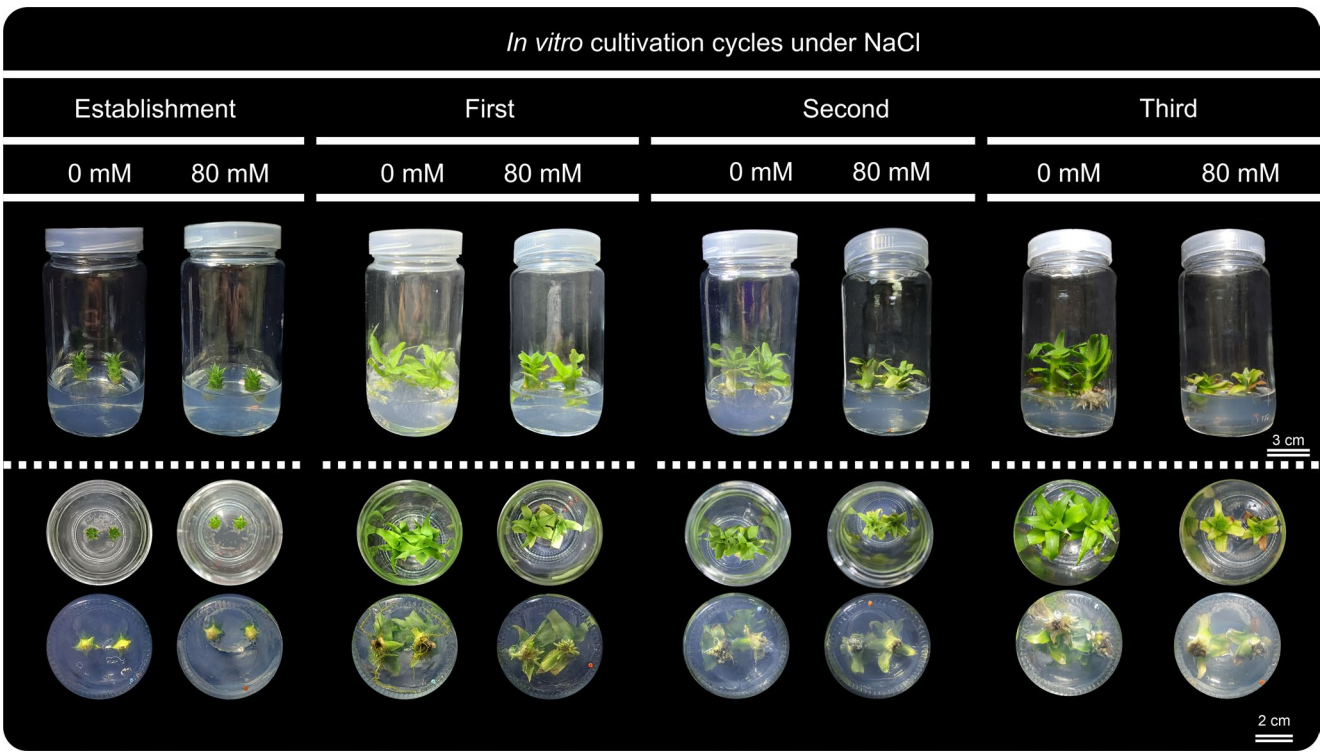

Fig. 1 *Ananas comosus* plantlets during three successive in vitro subcultures under 0 and 80 mM NaCl. Representative images correspond to the final subculture

Table 1 Growth parameters of *Ananas comosus* during successive in vitro subcultures under two salinity concentrations (0 and 80 mM NaCl)

Growth parameters	First cultivation		Second cultivation		Third cultivation	
	NaCl					
	[0 mM]	[80 mM]	[0 mM]	[80 mM]	[0 mM]	[80 mM]
Length of the aerial part (mm)	50.24 ± 4.42	41.62 ± 1.83*	55.00 ± 4.55	36.60 ± 2.25*	65.10 ± 1.69	36.10 ± 2.68*
Root length (mm)	31.95 ± 5.52	8.10 ± 0.96*	13.60 ± 3.18	5.40 ± 1.36*	0.84 ± 0.02	0.27 ± 0.04*
Rosette base diameter (mm)	10.34 ± 1.16	14.66 ± 1.57^{NS}	7.14 ± 0.45	8.41 ± 0.85^{NS}	7.88 ± 0.39	7.50 ± 0.45^{NS}
Number of leaves	16 ± 0.43	13 ± 0.64*	18 ± 2.20	14 ± 0.54^{NS}	21 ± 0.99	12 ± 0.68*
Plantlet fresh mass (mg)	1503 ± 141	1383 ± 95^{NS}	1630 ± 230	1760 ± 150^{NS}	2280 ± 84	1250 ± 138*
Plantlet dry mass (mg)	139 ± 13	142 ± 13^{NS}	180 ± 37	223 ± 23^{NS}	250 ± 7	211 ± 24^{NS}
Root fresh mass (mg)	169 ± 19	323 ± 44*	320 ± 43	313 ± 30^{NS}	1260 ± 51	581 ± 68*
Root dry mass (mg)	14 ± 1.7	21.6 ± 0.65*	40 ± 4.6	39 ± 4.3^{NS}	112 ± 4.45	67 ± 9.23*
Leaf area (cm^2)	38.91 ± 6.40	22.44 ± 3.19*	30.27 ± 6.29	24.93 ± 4.88^{NS}	50.61 ± 2.61	29.01 ± 2.20*

Data are means ± standard error (n=5). Asterisks indicate significant differences between treatments (*$P \leq 0.05$ two−tailed t−test) and NS, non−significant.

Table 2 Physiological parameters of *Ananas comosus* during successive in vitro subcultures under two salinity concentrations (0 and 80 mM NaCl)

Physiological parameters	First cultivation		Second cultivation		Third cultivation	
	NaCl					
	[0 mM]	[80 mM]	[0 mM]	[80 mM]	[0 mM]	[80 mM]
F_0	6590 ± 216	6839 ± 455^{NS}	6717 ± 237	5698 ± 216*	6806 ± 252	3306 ± 499*
F_m	29,069 ± 1033	23,561 ± 2126*	30,423 ± 1148	19,926 ± 1952 *	30,492 ± 622	5985 ± 977*
F_v	22,480 ± 852	16,722 ± 1704*	23,706 ± 1010	14,227 ± 1800*	23,686 ± 375	2678 ± 635*
F_v/F_m	0.77 ± 0.003	0.70 ± 0.012*	0.77 ± 0.008	0.70 ± 0.01*	0.78 ± 0.003	0.42 ± 0.05*
RC/ABS	1.39 ± 0.02	0.94 ± 0.07*	1.20 ± 0.13	1.1 ± 0.12^{NS}	1.42 ± 0.06	0.34 ± 0.08*
F_v/F_0	3.42 ± 0.07	2.45 ± 0.13*	3.54 ± 0.13	2.48 ± 0.24*	3.49 ± 0.07	0.82 ± 0.20*
PI	2.32 ± 0.10	1.08 ± 0.02*	2.06 ± 0.42	1.48 ± 0.37^{NS}	2.62 ± 0.19	0.17 ± 0.09*
Chlorophyll *a* ($\mu g\ cm^{-2}$)	26.31 ± 1.71	19.23 ± 1.22*	69.44 ± 2.41	40.58 ± 7.14*	69.58 ± 7.90	16.26 ± 1.04*
Chlorophyll *b* ($\mu g\ cm^{-2}$)	8.46 ± 0.55	5.81 ± 0.37*	20.69 ± 0.90	11.06 ± 2.21*	20.77 ± 3.15	3.31 ± 0.26*
Carotenoids ($\mu g\ cm^{-2}$)	4.67 ± 0.48	3.01 ± 0.16*	13.63 ± 0.57	9.08 ± 1.28*	13.94 ± 1.17	4.07 ± 0.13*
Total chlorophyll ($\mu g\ cm^{-2}$)	34.77 ± 1.42	25.04 ± 1.55*	90.12 ± 3.29	51.64 ± 9.36*	90.35 ± 11.05	19.57 ± 1.30*
Chlorophyll *a*/*b*	3.18 ± 0.32	3.31 ± 0.05^{NS}	3.36 ± 0.04	3.69 ± 0.08*	3.41 ± 0.10	4.96 ± 0.11*
Total chlorophyll/carotenoids	7.73 ± 0.73	8.41 ± 0.68^{NS}	6.61 ± 0.07	5.65 ± 0.20*	6.41 ± 0.21	4.80 ± 0.25*

Data are means ± standard error (n=5). Asterisks indicate significant differences between treatments (*$P \leq 0.05$ two−tailed t−test) and NS, non−significant. F_0: Initial fluorescence, F_m: maximum fluorescence, F_v: variable fluorescence, F_v/F_m: maximum quantum yield of PSII, RC/ABS: density of active reaction centers of photosystem II, F_v/F_0: maximum primary efficiency of photosystem II, PI: photosynthetic performance index

Salinity priming in vitro modulates photosynthetic performance and morphological characteristics under salt stress ex vitro

Salinity stress during acclimatization did not affect chlorophyll *a* fluorescence parameters in any priming treatment (Fig. 2). However, non-primed seedlings showed 15% higher F_0 than primed ones in both NaCl concentration (Fig. 2 A). Non-primed plants also maintained relatively stable F_m (Fig. 2B), F_v (Fig. 2C), and RC/ABS (Fig. 2E). The F_v/F_m remained approximately at 0.77 in both control and salinity treatments (Fig. 2D). In contrast, primed seedlings had 6% higher F_v/F_0 compared to non-primed plants regardless of salinity (Fig. 2F). No effect on PI was observed (Fig. 2G).

Gas exchange parameters were significantly affected by the interaction between salinity and priming (Fig. 3). In non-primed plants, salinity reduced net CO_2 assimilation rate (*A*) by 68%, stomatal conductance (g_s) by 60%, and transpiration rate (*E*) by 55% compared with the control (Fig. 3A, B, D). In primed plants, the reductions were less pronounced for *A* (48%), g_s (58%), and *E* (58%). Non-primed plants under salinity showed a 7% increase in intercellular CO_2 concentration (*Ci*) and *Ci/Ca* ratio relative to the control, whereas primed plants exhibited reductions of 13% in both parameters under salinity (Fig. 3C, E). The decrease in g_s, *E*, and *Ci/Ca* under salinity is consistent with stomatal limitation to CO_2 assimilation, particularly in primed plants. However, carboxylation efficiency (*A/Ci*) declined markedly under

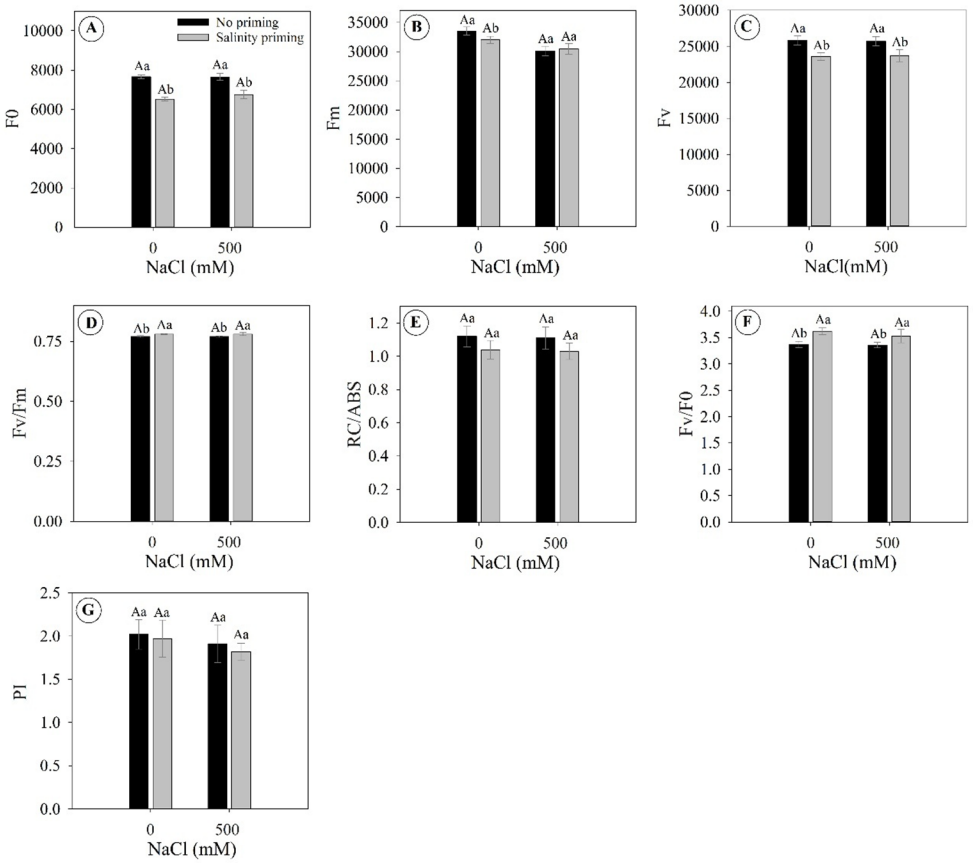

Fig. 2 Chlorophyll a fluorescence parameters of non-primed and salinity-primed *Ananas comosus* seedlings cultivated for 35 days under two salinity concentrations (0 and 500 mM NaCl). Data are means ± SE ($n = 11$). Uppercase letters indicate significant differences between NaCl concentrations within each priming treatment, whereas lowercase letters indicate differences between priming treatments within each NaCl concentration (Tukey's test; $P \leq 0.05$)

salinity in both treatment groups, decreasing by 69% in non-primed plants and by 40% in primed plants relative to their respective controls (Fig. 3F). Notably, under 500 mM NaCl, primed plants exhibited 56% higher *A/Ci* values than non-primed plants, indicating partial preservation of carboxylation efficiency under saline stress. These results suggest that, in addition to stomatal constraints, non-stomatal limitations contributed to the reduction in photosynthetic performance. Intrinsic water-use efficiency (A/g_s) decreased by 45% in non-primed plants under salinity, whereas primed plants maintained values statistically similar to their respective control (Fig. 3G).

Non-primed plants under salinity had a 7% increase in intercellular CO_2 concentration (C_i) and intercellular to ambient CO_2 concentration ratio (C_i/C_a) compared to control plants, while primed plants showed a 13% decrease in both parameters (Fig. 3 C and E). Carboxylation efficiency (A/C_i) decreased by 69% in non-primed plants under salinity, but this decrease was milder (40%) in primed plants. Plants under salinity showed a 33% decrease in A/C_i compared to non-primed plants (Fig. 3F). Salinity also reduced the intrinsic water-use efficiency (A/g_s) by 45% in non-primed seedlings (Fig. 3G). These results indicate that salt priming mitigated the detrimental effects of salinity on photosynthetic performance by improving the coordination between CO_2 diffusion and carbon assimilation, thereby maintaining higher carboxylation efficiency under stress conditions.

Salinity did not affect the chlorophyll *a*/b ratio (Fig. 4C) or the total chlorophyll to carotenoid ratio (Fig. 4F). However, non-primed plants exposed to salinity showed higher chlorophyll *a* (39%, Fig. 4A), chlorophyll *b* (38%, Fig. 4B), total chlorophyll (39%, Fig. 4D), and total carotenoid (46%, Fig. 4E) levels compared with the control. Rather than indicating enhanced photoprotective capacity, this apparent increase may reflect a concentration effect associated with salt-induced morphological adjustments, such as reduced leaf expansion, increased tissue thickness, or smaller cell size, resulting in higher pigment content when expressed on a tissue basis. Therefore, pigment accumulation alone should be interpreted cautiously and not as direct evidence of improved stress protection. This apparent increase may reflect a concentration effect rather than enhanced pigment biosynthesis, potentially resulting from reduced leaf expansion and/or smaller cell size under salt stress, which would increase pigment concentration on a tissue mass basis.

Salinity altered plant morphology depending on priming treatment. Under control conditions, primed plants exhibited reduced overall growth compared with non-primed plants. Under salinity, non-primed plants developed visible leaf necrosis and paler leaves, whereas primed plants also

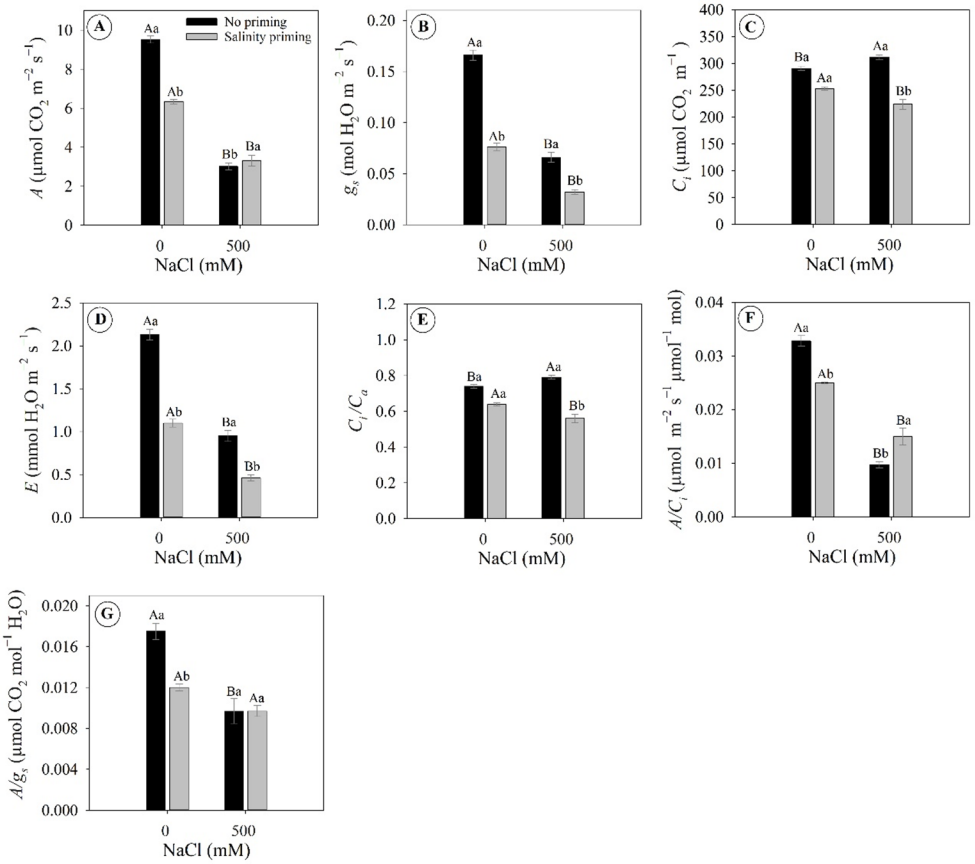

Fig. 3 Gas exchange parameters of non-primed and salinity-primed *Ananas comosus* seedlings cultivated for 35 days under two salinity concentrations (0 and 500 mM NaCl). Data are means ± SE ($n = 11$). Uppercase letters indicate significant differencesbetween NaCl concentrations within each priming treatment, whereas lowercase letters indicate differences between priming treatments within each NaCl concentration (Tukey's test; $P \leq 0.05$)

showed reduced growth, although with less pronounced visual damage. The increase in root length observed in non-primed plants under salinity should be interpreted cautiously, as it was accompanied by a reduction in root dry mass, suggesting a shift toward root elongation rather than enhanced root biomass accumulation. Overall, the morphological responses indicate distinct acclimation patterns between treatments rather than unequivocal growth advantages associated with priming (Fig. 5).

Salinity did not affect shoot length or rosette diameter (Fig. 5B and F), but non-primed plants were 106% longer and 126% larger than primed ones. Root length increased 37% under salinity in non-primed plants (Fig. 5C). However, salinity reduced leaf number (18%), rosette base diameter (44%), plantlet dry mass (41%), root dry mass (57%), leaf area (38%), and relative water content (31%) in non-primed plants, while primed plants were less affected (Fig. 5D, E, G, H, I and J).

Discussion

The present results indicate that in vitro salinity priming altered subsequent physiological responses of *A. comosus* under *ex vitro* salt stress, partially attenuating the decline in apparent carboxylation efficiency and modulating pigment responses. However, these physiological adjustments were accompanied by reduced plant growth under both control and saline conditions, indicating that improved acclimation responses did not translate into greater biomass accumulation. Similar priming-induced photosynthetic adjustments have been reported in other species and are consistent with priming-like responses suggestive of stress memory and enhanced physiological plasticity (Karalija et al. 2025; Sghaier-Hammami et al. 2023). However, direct confirmation of stress memory would require targeted molecular or epigenetic analyses, as well as assessment of plant performance following a controlled recovery period. The less pronounced reduction in carboxylation efficiency (A/C_i) in primed plants compared with non-primed plants suggests that salt priming partially preserved the biochemical capacity for carbon assimilation under saline conditions. Since A/C_i reflects the efficiency of CO_2 fixation independent of stomatal limitations, this response indicates that priming may have mitigated non-stomatal constraints associated with salinity, such as reduced Rubisco activity, impaired regeneration of photosynthetic intermediates, or damage to the photosynthetic apparatus, thereby contributing to better photosynthetic performance under stress (Molassiotis et al. 2010; Nasrallah et al. 2022).

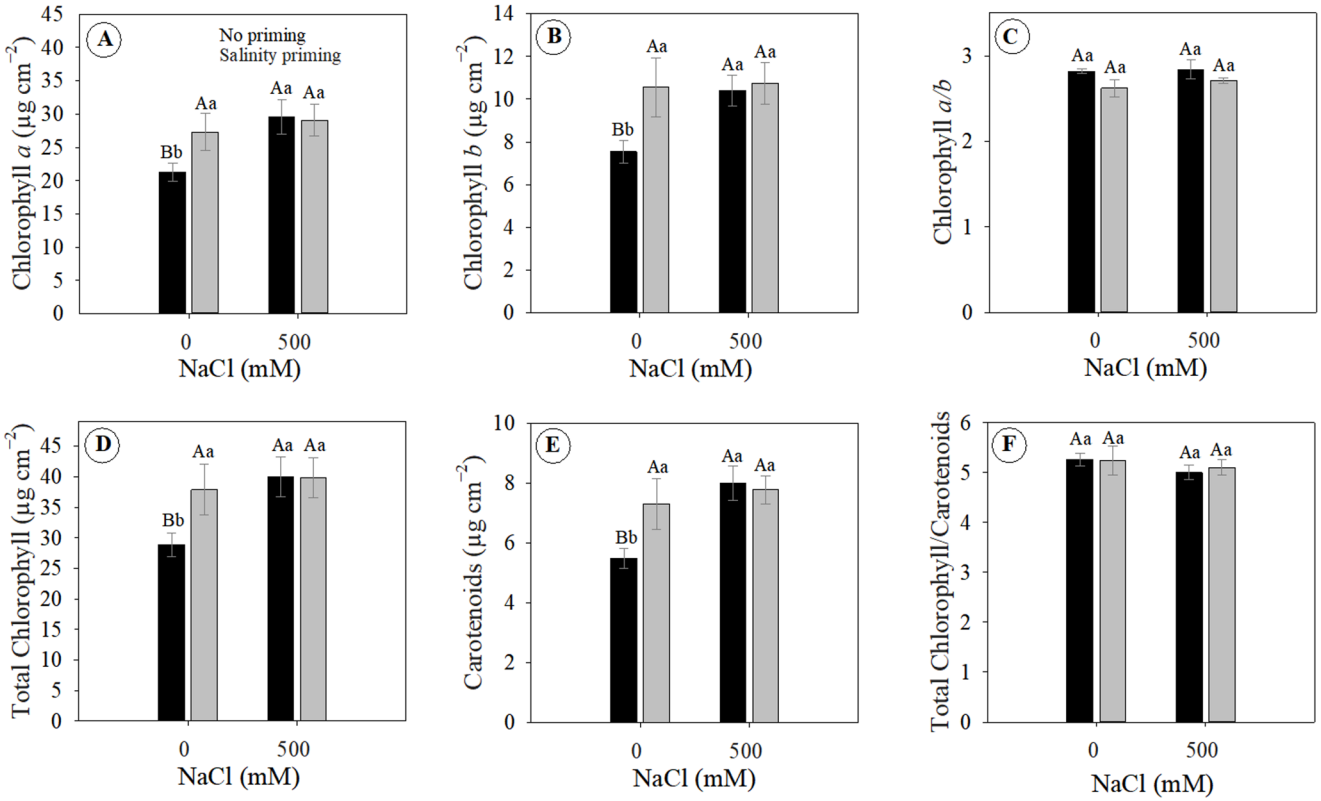

Fig. 4 Photosynthetic pigment contents of non-primed and salinity-primed *Ananas comosus* seedlings cultivated for 35 days under two salinity concentrations (0 and 500 mM NaCl). Data are means ± SE ($n = 4$). Uppercase letters indicate significant differences between NaCl concentrations within each priming treatment, whereas lowercase letters indicate differences between priming treatments within each NaCl concentration (Tukey's test; $P \leq 0.05$)

Despite these physiological adjustments, the absence of proportional gains in net CO_2 assimilation and biomass indicates that the partial preservation of photosynthetic performance was insufficient to sustain growth under salinity. The reduction in stomatal conductance and transpiration under salinity indicates that stomatal restriction contributed to the limitation of carbon assimilation, particularly in primed plants, where the decline in C_i/C_a suggests reduced CO_2 diffusion into the leaf. In contrast, the increase in C_i/C_a observed in non-primed plants under salinity, despite lower stomatal conductance, indicates that non-stomatal limitations also played an important role, likely reflecting impaired carbon fixation capacity. Together, these contrasting responses suggest that salinity constrained photosynthesis through different combinations of stomatal and non-stomatal limitations depending on the priming history. Simultaneously, maintenance of chlorophyll and carotenoid pools indicates that priming promoted photoprotective stabilization of the photosynthetic apparatus, likely preserving PSII integrity and limiting oxidative pigment degradation (Zandi and Schnug 2022).

However, the reduced growth observed in primed plants may suggest a potential trade-off associated with maintaining stress-responsive physiological adjustments. One possible explanation is that the sustained activation of protective and repair mechanisms could impose additional energetic demands, potentially reducing carbon allocation to biomass production (Badavath and Saski 2026). Nevertheless, this interpretation remains hypothetical, as the metabolic processes underlying such trade-offs were not directly evaluated in the present study. This potential trade-off may have been further intensified by the severity of the salinity treatment, as 500 mM NaCl represents an extreme stress condition likely to shift plant responses toward survival and maintenance rather than growth. Under such conditions, even partial physiological acclimation may not be sufficient to promote biomass accumulation, as substantial energetic and metabolic resources are likely redirected toward sustaining cellular homeostasis under severe ionic and osmotic stress (Nigam et al. 2026).

In the present study, the observed divergence between partial physiological acclimation and reduced biomass accumulation suggests that improved stress-responsive physiological adjustments do not necessarily translate into enhanced growth under severe salinity. Although priming has frequently been associated with improved biomass

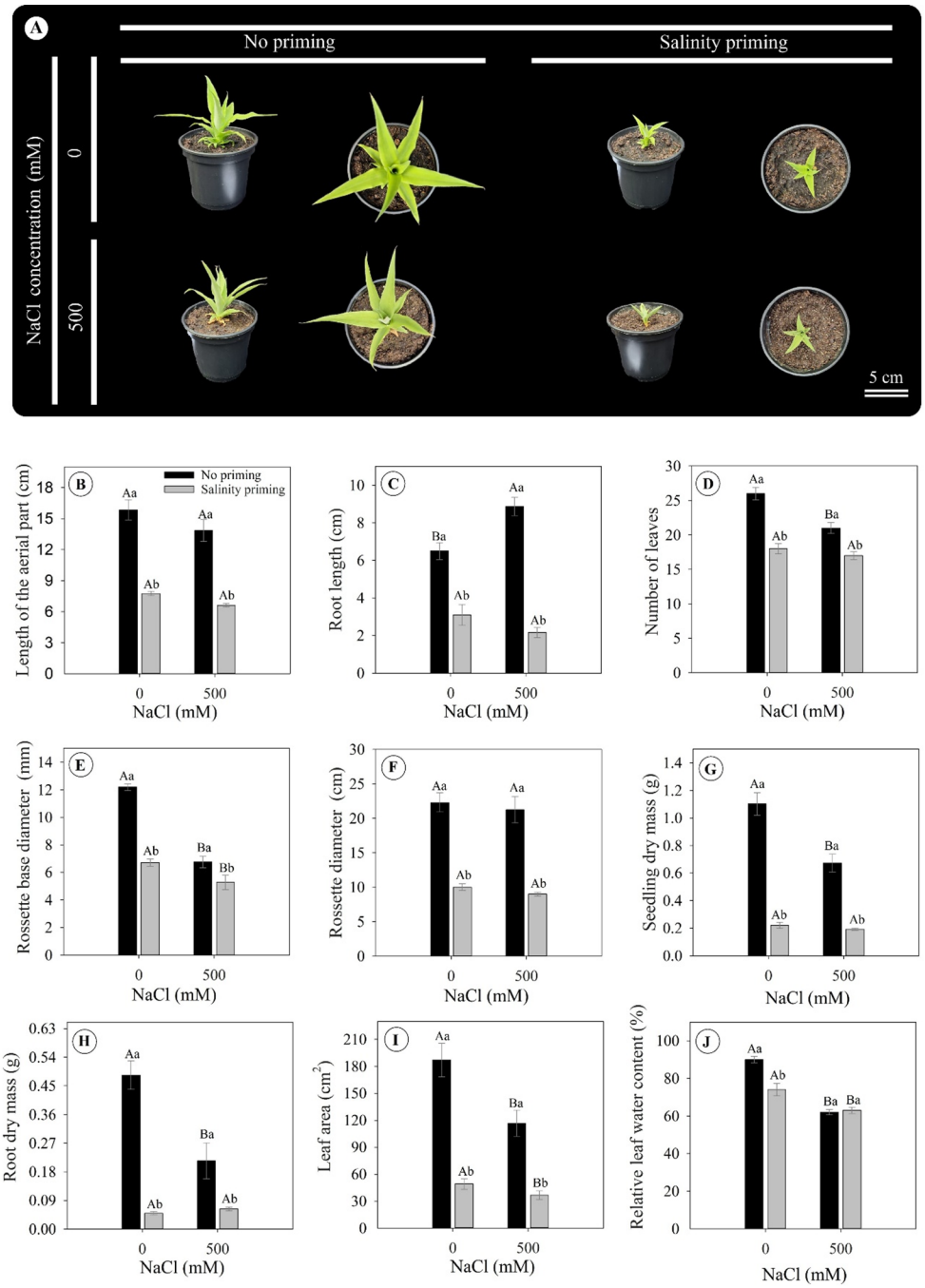

Fig. 5 Growth parameters of non-primed and salinity-primed *Ananas comosus* seedlings cultivated for 35 days under two salinity concentrations (0 and 500 mM NaCl). Data are means ± SE ($n = 5$). Uppercase letters indicate significant differences between NaCl concentrations within each priming treatment, whereas lowercase letters indicate differences between priming treatments within each NaCl concentration (Tukey's test; $P \leq 0.05$)

production and yield under moderate stress conditions (Sahoo et al. 2025), these benefits appear to be strongly context-dependent. Under extreme stress, such as the 500 mM NaCl imposed here, the capacity of priming-induced acclimation to support growth may be limited, potentially due to the prioritization of maintenance and survival processes over biomass accumulation. However, because the present study evaluated only a single *ex vitro* salinity level, it is not possible to define the threshold at which priming ceases to confer growth benefits. Future studies employing a gradient of salinity levels will be important to determine the range over which priming promotes beneficial acclimation without compromising plant growth.

Conclusion

Under the severe *ex vitro* salinity imposed here, in vitro salinity priming partially attenuated the decline in apparent carboxylation efficiency and maintained pigment concentrations, but did not improve biomass accumulation in *Ananas comosus*. These results suggest a decoupling between

selected physiological adjustments and growth recovery under severe salt stress, in which the maintenance of stress-responsive acclimation **may impose** constraints on carbon allocation to biomass production.

Author contributions Conceptualization and supervision were performed by Felipe, S.H.S. and Corrêa, T.R. Data collection, investigation, and methodology were carried out by Silva-Moraes, V.K.O., Alves, G.L., Albuquerque, I.C., Pinheiro, J.F., Lima, A.S., Rivas, P.M.S. Formal analysis was performed by Silva-Moraes, V.K.O., Corrêa, T.R., Felipe, S.H.S., Batista, D.S., Figueiredo, F.A.M.M.A., Reis, F.O., Ferraz, T.M. The first draft of the manuscript was written by Silva-Moraes, V.K.O. All authors reviewed, edited, and approved the final version of the manuscript for publication.

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Declarations

Conflict of interest D.S.B. serves as an Associate Editor of the journal at the time of submission. However, this did not influence the peer review process or the final decision. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability The authors confirm that the data supporting the findings of this study are available in the article.

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